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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Penetration of mepacrine and quinine into both normal and parasitized erythrocytes increases with lowering of the pH, and does so to a greater extent with parasitized cells.

The pH dependence does not replace the chloride shift. It is concluded that penetration of schizonticidal drugs into parasitized erythrocytes is governed by the accumulation of non-dialysable fatty acids which lowers the internal pH. (over)

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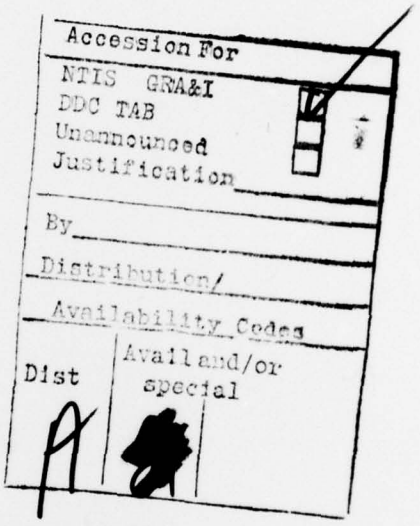
20. Fatty acids are implicated in the destruction of erythrocytes by malaria parasites. The buffering capacity of AA, AS and SS haemoglobin was therefore examined and found to be considerably increased with sickle cell haemoglobin. This agrees well with the increase in positive charge due to the amino acid mutation in one Hb-chain and could provide a simple explanation of the selective advantage of sickle-cell trait in man, by preventing or retarding the release of merozoites and the associated intravascular haemolysis.



IN VITRO RESEARCH INTO THE ACTION MECHANISM OF ANTIMALARIAL DRUGS AND RELATED PROBLEMS OF IMMUNITY

Final Report

July 1977

by

H. Laser, M. D., Sc. D., Ph. D.

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1. Drug Penetration

It has previously been shown that the penetration of mepacrine and quinine into both normal and parasitized erythrocytes increases with lowering of the outside pH, but to a greater extent into parasitized cells, making it appear as if the intraerythrocyte pH of the parasitized cells is of the order of one pH unit lower than that in normal cells.

It was, therefore, tempting to assume that the drug acts as or replaces the chloride in the mechanism of the physiological chloride shift. That, however, was found not to be the case. On exposing erythrocytes to Mepacrine in presence of ^{36}Cl labelled chloride in phosphate buffers between pH 5-8, the drug while taken up by the erythrocytes with decreasing pH values, did not affect the simultaneous increase in chloride uptake by the erythrocytes (Table I). The experimental series, while still to be extended, allows the conclusion that the relative increase of penetration of schizonticidal drugs into parasitized erythrocytes is governed by the accumulation of intraerythrocytic non-dialysable fatty acids. This is in agreement with our previous findings on the action mechanism of Quinine, in fact it is the logical corollary.

2. Abnormal Haemoglobins

The second series of experiments deals with some properties of abnormal haemoglobins such as sickle cell Hb which have a bearing on malaria. A short paper of our first results is included. (H. Laser and R. Klein, 1977).

We are extending these experiments to other haemoglobins, depending on their availability, and to the study of the composition of their membranes, the phospholipid content of which, unfortunately, greatly depends on the diet of the individual. We hope soon to get blood samples from a brother and sister or husband and wife, living under identical conditions, one of whom only is sickling. We have shown that the haemolytic activity of fatty acids may be implicated in the destruction of erythrocytes by malaria parasites (Laser et al., 1975, a, b). Under normal conditions free fatty acids are buffered by albumin in the serum and haemoglobin within the erythrocyte. During a malarial infection, however, the presence of the Plasmodium leads to an increase in both serum and erythrocyte fatty acid concentrations which may exceed the buffering capacity of the available protein. We have, therefore, examined the fatty acid-buffering capacity of normal and sickle cell haemoglobin and found that haemolysis by added oleic acid is delayed by the presence of haemoglobin S, as compared with the same concentration of haemoglobin A, with the homozygous haemoglobin (SS) being more effective than the heterozygous (AS) material. Haemoglobin from patients with SC disease was also found to delay haemolysis and appears to be more effective than homozygous sickle-cell haemoglobin.

A possible molecular interpretation of these results may be based on the well-known differences in electrophoretic mobility, due to the amino acid mutation at the sixth position of the β residue (Valine or lysine respectively in place of glutamic acid). This could provide a simple explanation of the selective advantage of sickle-cell train (AS) in man,

by preventing or retarding the release of merozoites and the associated intravascular haemolysis.

3. Cultivation

It is unavoidable that basic studies of the Malaria problem must ultimately lead to two aspects which have so far been recalcitrant, namely, the cultivating (en masse) of the parasites *in vitro* (which may be purely technical) and the field of Immunity, which would be helped by the former. My approach to this has been catalysed by a statement by Garnham (1963), quoted in Section A, para. 4, to the effect that, at least in falciparum, shziogony takes place inside some organs but not in the circulating blood.

It seems possible, therefore, that the relative failure, so far, to grow the parasites to a desired considerable degree may be due to the absence of the right milieux for the 'hatching' of the merozoites, namely, a short arrest inside an organ. I have, therefore, embarked on devising a method of *in vitro* blood circulation over some length of time, say 2 - 3 days, where at given times the blood is short circuited through an organ (lung, heart) connected into the circulation. The technical difficulties are considerable but can be overcome.

4. Immunity

Lastly, some immunological experiments are currently in progress, based on the following consideration:

(1) Some parasites prefer old, others young erythrocytes (reticulocytes), (Cox 1974). They must, therefore, be able to distinguish between them, i.e. to recognise them (cf. also Duffy phenotypes). Furthermore, the work of Bannister et al., (1975) on the entry of the parasites into the erythrocytes makes it not improbable that the parasites connect with and recognise a receptor in the cell membrane.

We are, therefore, trying to affect the erythrocyte membrane in such a way as to inhibit the entry of parasites. This is attempted by the adsorption of (non-haemolytic) erythrocyte antibody-fractions prepared from the serum of sheep or rabbits which had been injected with parasitized mouse erythrocytes. The experiments have only just begun in collaboration with our Department of Immunology and with Professor F. E. G. Cox, London, and I am not able yet to report meaningful results.

(2) We have been able to obtain malaria placental antigen (falciparum) and immune sera from the Medical Research Council Laboratories in Gambia. First results are encouraging.

(a) The antigen seems to contain a diglyceride. This would confirm our former findings (with knowlesi and Berghei) of a parasitic phospholipase. We await confirmation of the

diglyceride by mass spectrometry.

(b) We have found interaction between antigen and serum by diffusion on an agar plate (i.e. a precipitate). This indicates a soluble antigen and makes it appear that the operative antigen is not "the parasite", but a parasite-metabolite (Lipase??)

Work on the fractionation of the crude placental antigen is in progress.

TABLE I

Uptake of ^{36}Cl by erythrocytes in the presence of 2×10^{-4}M Mepacrine

pH	CAP %	VOL µl	C/V
5	7.6	5.447	1.395
6	3.82	5.326	.717
7	2.15	3.958	.543
8	2.10	3.282	.639

Haematocrit 4.54%

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